DALHOUSIE

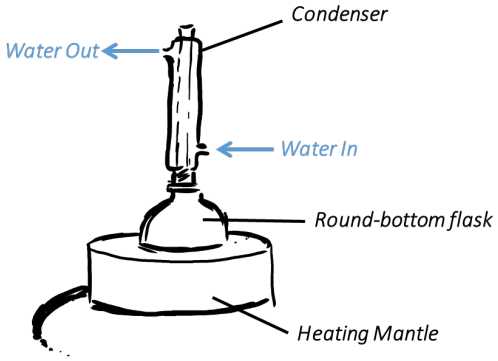
2016 Protocols

Steam Distillation

Purpose:
To isolate terpenes found in pine tree oleoresin with minimal decomposition, and analyze the collected residue.

Required Materials:
- Pine tree oleoresin (PTO)
- Condenser
- Stillhead
- Thermometer
- Heating mantle
- Boiling chips
- Round-bottom flask
- Glass wool
- Seperatory funnel
- Graduated cylinder
- Diethyl ether (OEt_2)
- Hexanes
- Dichloromethane (CH_2Cl_2)
- Anhydrous sodium sulfate ($Na_2SO_{4(s)}$)
- Saturated NaCl solution

Procedure:
1. Place PTO in a hot water bath (to liquefy and ease manipulation)
2. Pour desired mass of warm PTO into round-bottom flask.
3. Add 75 mL distilled water for every gram of PTO added
4. Add several boiling chips (more should be added over the course of the distillation to prevent bumping).



Assemble apparatus as depicted in

5. **Figure 1**. Note that the round-bottom flask should be wrapped in glass wool to minimize heat dissipation into the air.

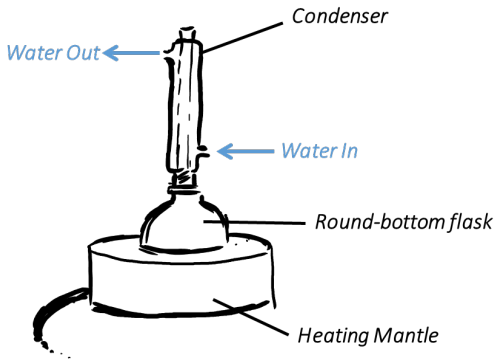


Figure 1

6. Reflux for 30 min.
7. Re-assemble apparatus as depicted in **Figure 2**.
8. Collect distillate for 1 hr per gram of PTO added. (The resulting distillate should be cloudy water, or clear water with small droplets floating on top). The thermometer should read slightly below 100°C (96-98).
9. After sufficient time and distillate is collected, extract the distillate using a seperatory funnel according to the following scheme:
    a. Approximately 10 mL OEt_2 per 40 mL distillate, twice.
    b. Approximately 20 mL Hexanes per 40 mL distillate, twice.
    c. Approximately 20 mL CH_2Cl_2 per 40 mL distillate, twice.

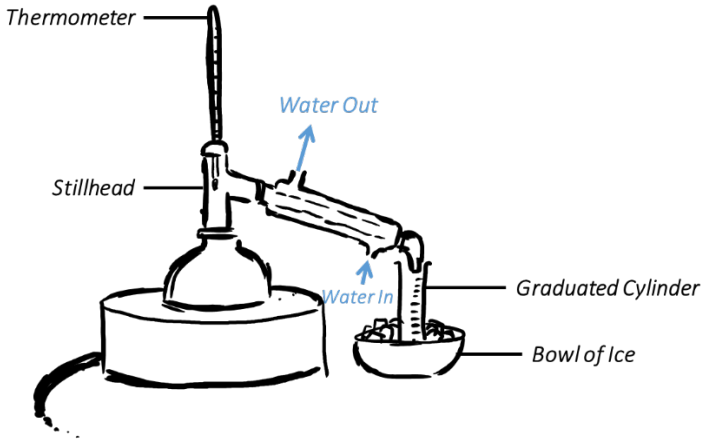


Figure 2

10. Combine all organic layers, and then wash with 20 mL sat. $NaCl_{(aq)}$ using a seperatory funnel.
11. Dry organic layer using anhydrous $Na_2SO_{4(s)}$, then filter off the solid matter.
12. Gently evaporate to dryness, either by low heating or by using a rotary evaporator (note that the solvents have very low boiling points, so not much heat is required).

13. A pine-smelling residue should be obtained (the yield likely dependent on many different variables).
14. Collect a small quantity of the residue, dilute x20 using CH_2Cl_2. This sample can be analyzed with GC/MS using the following parameters:
    a. We used a SLB-5ms fused silica capillary column, 30m x 0.25 mm i.d., film thickness 0.25 micrometeres. The column temperature started at 70°C, and increased at 2°C/min to 200°C. The carrier gas, Helium, was delivered at 1.70 mL/min (split ratio is 1:10). Electron ionization mass spectrometry was used in order to identify terpenes by their fragmentation patterns.